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1: Biochemistry. 1982 Sep 14;21(19):4535-40. Related Articles, Links

Reversible denaturation of Aequorea green-fluorescent protein: physical separation and characterization of the renatured protein.

Ward WW, Bokman SH.

The green-fluorescent protein (GFP) that functions as a bioluminescence energy transfer acceptor in the jellyfish Aequorea has been renatured with up to 90% yield following acid, base, or guanidine denaturation. Renaturation, following pH neutralization or simple dilution of guanidine, proceeds with a half-recovery time of less than 5 min as measured by the return of visible fluorescence. Residual unrenatured protein has been quantitatively removed by chromatography on Sephadex G-75. The chromatographed, renatured GFP has corrected fluorescence excitation and emission spectra identical with those of the native protein at pH 7.0 (excitation lambda max = 398 nm; emission lambda max = 508 nm) and also at pH 12.2 (excitation lambda max = 476 nm; emission lambda max = 505 nm). With its peak position red-shifted 78 nm at pH 12.2, the Aequorea GFP excitation spectrum more closely resembles the excitation spectra of Renilla (sea pansy) and Phialidium (hydromedusan) GFPs at neutral pH. Visible absorption spectra of the native and renatured Aequorea green-fluorescent proteins at pH 7.0 are also identical, suggesting that the chromophore binding site has returned to its native state. Small differences in far-UV absorption and circular dichroism spectra, however, indicate that the renatured protein has not fully regained its native secondary structure.

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